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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C08B 37/06, A23L 2/38 — A1

(11) International Publication Number: **WO 99/03892**

(43) International Publication Date: 28 January 1999 (28.01.99)

(21) International Application Number: PCT/NL98/00407

(22) International Filing Date: 14 July 1998 (14.07.98)

(30) Priority Data:
1006602 16 July 1997 (16.07.97) NL

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NOVEL PECTIN

(57) Abstract

Pectin, obtained by extraction of chicory or Jerusalem artichoke material, having the following characteristics: an average molecular weight of 30–2000 kDa, an arabinose content of 2–30 mol %, a galactose content of 5–20 mol %, a rhamnose content of 1–15 mol %, a galacturonic acid content of 30–85 mol %, a degree of methylation (of the galacturonic acid units) of 20–80 %, a degree of acetylation (of the galacturonic acid units) of 1–50 %. The novel pectin is an excellent emulsifier and can also be used as a binder, as a surfactant and as dietary fibre.

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Novel pectin

The invention relates to a novel pectin having improved emulsifying and surfactant properties.

Pectin is a naturally modified polysaccharide, essentially made up of regions containing polygalacturonic acid chains and regions containing chains which consist alternately of rhamnose units and galacturonic acid units, with side chains which mainly contain arabinose units and galactose units. The acid groups of the galacturonic acid units have been partially esterified with methanol and some of the hydroxyl groups thereof have been acetylated. It has a molecular weight of the order of 30,000 to 2,000,000. Pectin is widely used in foods, in particular as a thickener, gelling agent and emulsifier. Pectin is usually obtained from apples and citrus fruits.

Pectin originating from sugar beet is described in EP-A-426434. It is obtained by acid or basic extraction of sugar beet material at high temperature (about 160 °C), followed by centrifuging and ultrafiltration. It differs from apple pectin and citrus pectin in that it has, inter alia, a relatively low molecular weight, a high degree of acetylation and a low viscosity. Diverse food applications are proposed, including use as an agent for lowering cholesterol.

Extraction of pectin from sugar beet at about 95°C and pH 3 is described in DE-C-4313549. Precipitate formation is not employed, as a result of which a pectin having a large number of short chains is obtained. It is proposed as a foam stabiliser.

It has now been found that a pectin which has a number of characteristics which make it more interesting than other pectins can be obtained industrially from roots and pulp of Asteraceae (Compositae) plants, especially chicory and Jerusalem artichoke, most particularly chicory.

Chicory is a crop plant that is cultivated in its various varieties as a vegetable ("witloof chicory") and as a source of inulin. Jerusalem artichoke is also cultivated as a source of inulin. They are totally unrelated to other crops from which pectin is obtained. As can be seen from the following list, in the classification of the plant kingdom there is a correspondence with sugar beet only at the level of the class (that of the dicotyledons); the same applies to the citrus fruits and the apple

	Sugar beet	*Chicory*	*Jerusalem artichoke*
Sub–kingdom	Cormophyta	Cormophyta	Cormophyta
Division	Spermatophyta	Spermatophyta	Spermatophyta
Sub–division	Angiospermae	Angiospermae	Angiospermae
Class	Dicotyledoneae	Dicotyledoneae	Dicotyledoneae
Sub–class	Caryophyllidae	Asteridae	Asteridae
Order	Caryophyllales	Asterales	Asterales
Family	Chenopodiaceae	Compositae (Asteraceae)	Compositae (Asteraceae)
Genus	Beta	Cichorium	Helianthus
Species	*B. vulgaris*	*C. intybus*	*H. tuberosus*

Chicory and Jerusalem artichoke pectin can be obtained by extraction of chicory roots or pulp and Jerusalem artichoke tubers or pulp, respectively (optionally after washing, pressing and comminuting) with water (about 10–30 times the weight of chicory) at elevated temperature (at, for example, 70–160 °C) under acid conditions (pH 1–6) for a few minutes to a few hours. Further working up can be effected by, for example, microfiltration, optionally followed by ultrafiltration, and spray–drying or freeze–drying. Instead of ultrafiltration, the pectin can be precipitated by alcohol or alcohol/water (about 2:1), followed by filtration.

The Asteraceae root pectin thus obtained has properties as specified in the claims: an average molecular weight of 30–2000 kDa, an arabinose content of 2–30 mol % (especially 5–20 mol %), a galactose content of 5–20 mol % (10–15 mol %), a rhamnose content of 1–15 mol % (5–10 mol %), a galacturonic acid content of 30–85 mol % (50–80 mol %), a degree of methylation of the galacturonic acid units of 20–80 % (35–55 mol %), and a degree of acetylation of the galacturonic acid units of 1–50 % (1–30 mol %). In addition to the sugars mentioned, Asteraceae pectin also contains small amounts of glucose, mannose and xylose residues. In this context it can be pointed out that the composition is partly dependent on the method of extraction. For instance, with mild extraction (pH > 3 and/or T < 120 °C) the arabinose content can be higher than the indicated value; on extraction at pH 4 – 4.5, 1–5 % rhamnose, 30–60 % arabinose, 3–6 % galactose and 30–55 % galacturonic acid are found. The final molar weight distribution is dependent on the extraction method and can also be adjusted by the choice of the filters used and of whether or not to employ precipitation. Chicory and Jerusalem artichoke pectin also contains ferulic acid residues (0.01 – 0.5 % by wt).

Chicory (and Jerusalem artichoke) pectin according to the invention is further characterised by the presence of sesquiterpene lactones, such as laetucin and lactucopicrin and glucosides and other analogues thereof, at a total level of at least 20 μg/g, especially 40–300 μg/g. In particular, the level of lactucin–like compounds in chicory pectin is between 30 and 200 μg/g and the level of lactucopicrin is between 10 and 60 μg/g. Lactucin–like compounds include lactucin (9α–hydroxy–3–hydroxymethyl–7–methyl––11–methylene–13–oxatricyclo[9,3,0,$0^{2,6}$]trideca–3,6–diene–5,12–dione, systematic numbering and lactucin numbering deviate), 8–deoxylactucin and their 11β,13–dehydro derivatives as well as their glucosides; lactucopicrin is the p–hydroxyphenylacetate ester of lactucin. For comparison, the level of these compounds in beet pectin is below 8 μg/g and below 3 μg/g, respectively. The concentration of these sesquiterpene lactones can be determined by means of specific antibodies using e.g. enzyme–linked immunosorbent assay, or by means of high performance liquid chromatography as described by Peters and Van Amerongen (Z. Lebensm. Unters. Forsch. A (1997) 204: 189–193).

The Jerusalem artichoke pectin and especially the chicory pectin according to the invention have a low surface tension and a higher viscosity than beet pectin: the surface tension is preferably less than 60 mN/m, in particular less than 50 mN/m, for a 0.1 % by wt solution, and the viscosity is 100–300 mPa.s for a 2.5 % by wt solution. It does not have any foaming power (Ross & Miles test). It has excellent emulsifying and binding properties. The emulsion stability of chicory and Jerusalem artichoke pectin is at least as high as that of the same dosage of gum arabic or beet pectin. These pectins are also more effective than beet pectin as a binder and thickener. Chicory and Jerusalem artichoke pectin can also advantageously be used as a soluble dietary fibre.

The invention relates to chicory root pectin as such but also in combination with other chicory root constituents, in particular chicory proteins and inulin, and similarly with Jerusalem artichoke pectin. A mix preparation can contain, for example, 1–75 % by wt, in particular 2–25 % by wt, chicory or Jerusalem artichoke protein or inulin. Chicory and Jerusalem artichoke pectin can also advantageously be combined with other pectins, in particular citrus or apple pectin, or other emulsifiers, surfactants, solvents, binders or thickeners.

Example 1: Preparation of chicory root pectin

225 g chicory pulp was washed with 10 parts of demineralised water at 50 °C. The wash water was removed by pressing. The washed pulp was comminuted by shear treatment

to produce a slurry. The slurry was extracted with 6 litres of water (pH 1.5, 85 °C, 1 hour), after which the extract was filtered off (cut-off: 80-100 µm) and decanted. The supernatant liquor was thickened with the aid of a vacuum evaporator to a solids content of about 4 %. 60-80 % by vol. ethanol (96 %) was added to this concentrate, after which the precipitate was separated off and dried. The yield of chicory pectin with the preparation described is about 15 % based on solids.

The sesquiterpene lactone concentration was determined by dissolving two 0.5 g samples in 5 ml demi water containing cellulase (Onozuka) and Macerozym (1 mg/ml each), incubating for 2 h with mixing, centrifuging and using the supernatant in an ELISA test as described by Peters and Van Amerongen, Z. Lebensm. Unters. Forsch. A (1997) 204: 189-193, as follows.

Microtitre plate wells were coated overnight at 4°C with BSA-lactucin or BSA-lactucopicrin at 5 ng/100 µl PBS. After incubation, the wells were washes three times (350 µl/well) with PBS-Tween (0.1 M PBS, pH 7.5, 0.1 % Tween 20) and blocked with 200 l of PBS-BSA (0.1 % PBS, pH 7.5, 2 % BSA) at 37°C for 1 h. The plates were stored at -20°C until use. Before use, plates were washed three times with 350 µl of PBS-Tween to remove any excess of blocking agent. Then 50 µl of a serial dilution of pectin extracts was added to the wells in duplicate, followed by 50 µl of antibody at appropriate dilution. For the detection of lactucin-like lactones, a polyclonal antiserum (no. 455) was used, while for the detection of lactucopicrin, a monoclonal antibody (no. 4H10) was used. Serial dilutions of the purified lactones were used as standard. Plates were incubated at 37°C for 1 h and washed as described above. Then 100 µl of goat anti-rabbit IgG-AP or goat anti-mouse IgG-AP in a 1:1000 dilution in PBS was added to each well. After incubation at 37°C for 1 h, plates were washed and 200 µl of freshly prepared substrate buffer (10 mg p-nitrophenyl phosphate in 10 ml of a 50 mM carbonate buffer, pH 9.6, 0.5 mM $MgCl_2$) was added. The absorbance at 405 nm was determined in a BioRad, model 3550-UV, ELISA reader after 45 min incubation at 37°C. The BioRad Microplate Manager/PC Data analysis software was used to calculate sesquiterpene lactone concentrations in the samples using the standards. Each sample was measured in triplicate and the ELISAs were performed two to four times. The level of lactucin-like lactones was 121 ± 35 µg/g and the lactucopicrin level was 32 ± 18 µg/g (dry weight basis).

For two samples of beet pectin, the comparable level of lactucin-like lactones was 3.6 ± 3 µg/g and the lactucopicrin level was 1.6 ± 1 µg/g.

Example 2:

A stable lemon oil emulsion consists of:

Lemon oil	10.0 %
Chicory pectin	1.0 %
Ester gum	10.0 %
Sodium benzoate	0.2 %
Citric acid	0.2 %
Water	78.6 %

The emulsion is prepared by dissolving the chicory pectin in the water and the ester gum in the oil and homogenising these two phases using a "high shear" mixer. The sodium benzoate and the citric acid are added before homogenisation. The degree of emulsification is determined by determining the oil droplet size of the emulsion. The emulsion stability is determined by visual assessment of the emulsion, measuring, inter alia, the quantity of creamed oil.

The emulsion is stable for at least one month. A similar emulsion that contains the same quantity of commercial beet pectin (Genu BETA, Hercules, Copenhagen, DK) instead of chicory pectin starts to de-mix after one week.

Claims

1. Pectin, obtained by extraction of Asteraceae root material, having the following characteristics:
- an average molecular weight of 30–2000 kDa,
- an arabinose content of 2–30 mol %,
- a galactose content of 5–20 mol %,
- a rhamnose content of 1–15 mol %,
- a galacturonic acid content of 30–85 mol %,
- a degree of methylation (of the galacturonic acid units) of 20–80 %,
- a degree of acetylation (of the galacturonic acid units) of 1–50 %.

2. Pectin according to Claim 1, obtained by extraction of chicory or Jerusalem artichoke material, having one or more of the following characteristics:
- an arabinose content of 5–20 mol %,
- a galactose content of 10–15 mol %,
- a rhamnose content of 5–10 mol %,
- a galacturonic acid content of 50–80 mol %,
- a degree of methylation (of the galacturonic acid units) of 35–55 %,
- a degree of acetylation (of the galacturonic acid units) of 1–30 %.

3. Pectin, obtained by extraction of chicory or Jerusalem artichoke material, having the following characteristics:
- an average molecular weight of 30–2000 kDa,
- an arabinose content of 30–60 mol %,
- a galactose content of 3–6 mol %,
- a rhamnose content of 1–5 mol %,
- a galacturonic acid content of 30–55 mol %,
- a degree of methylation (of the galacturonic acid units) of 20–80 %,
- a degree of acetylation (of the galacturonic acid units) of 1–50 %.

4. Pectin according any one of Claims 1–3, further containing lactucin and lactucopicrin and/or glucosides and other analogues thereof, at a total level of at least 20 μg/g.

5. Pectin according any one of Claims 1–4, having one or more of the following characteristics:

- a surface tension of less than 60 mN/m, in particular less than 50 mN/m, for a 0.1 % by wt solution,
- a viscosity of 100–300 mPa.s for a 2.5 % by wt solution.

6. A pectin–containing preparation that contains a pectin obtained from chicory Jerusalem artichoke in combination with a pectin obtained from a citrus fruit or apple.

7. A pectin–containing preparation that contains a pectin obtained from chicory Jerusalem artichoke in combination with a protein obtained from chicory.

8. A pectin–containing preparation that contains a pectin obtained from chicory or Jerusalem artichoke in combination with inulin.

9. A method for the preparation of pectin, characterised in that chicory or chicory pulp or Jerusalem artichoke or pulp thereof is extracted with water at pH 1–6 (in particular 1.5 – 3) and 70–160 °C (in particular 95–140 °C) and is then isolated by filtration and/or precipitation steps.

10. Use of the pectin according to one of Claims 1–5 or of a preparation according to Claim 6 or 7 as an emulsifier.

11. Use of the pectin according to one of Claims 1–5 or of a preparation according to Claim 6 or 7 as a binder or thickener.

12. Use of the pectin according to one of Claims 1–5 or of a preparation according to Claim 6 as a surfactant.

13. Use of the pectin according to one of Claims 1–5 or of a preparation according to Claim 6 as a solvent assistant.

14. Use of the pectin according to one of Claims 1–5 or of a preparation according to Claim 6 or 8 as soluble dietary fibre.

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/NL 98/00407

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08B37/06 A23L2/38

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C08B A23L

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DR. M.B.KATAN ET AL.: "Analyse van het totale voedingsvezelgehalte en van het pectine-aandeel hierin in Nederlandse voedingsmiddelen." VOEDING, vol. 43, no. 5, 1982, pages 153-160, XP002058090 see page 156; table 3 ---	1-7
A	DATABASE WPI Week 8118 Derwent Publications Ltd., London, GB; AN 32047D XP002084906 & SU 757 541 A (AS KIRG ORG CHEM) , 23 August 1980 --- -/--	1

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